PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P 63658	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/07551	International filing date *(day/month/year)* 11.07.2003	Priority date *(day/month/year)* 12.07.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/08		
Applicant BLASTICON BIOTECHNOLOGISCHE FORSCHUNG GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.10.2003	19.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer van Heusden, M Telephone No. +49 89 2399-8145

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/07551

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-65 as originally filed

Claims, Numbers

1-26 received on 21.06.2004 with letter of 18.06.2004

Drawings, Sheets

1/20-20/20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: ; which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-26
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

   see separate sheet

Additional remarks to section V:

1. Novelty (Article 33(2) PCT)

1.1 The present application discloses a process for the preparation of transplant acceptance inducing cells (TAIC) of monocytic origin by cultivating the blood-derived monocytes in the presence of M-CSF and γ-IFN. It further relates to the resulting TAIC and their therapeutic applications. It also relates to a process of generating $CD4^+CD25^+$ regulatory T-lymphocytes by co-cultivation of T-lymphocytes with said TAIC. It finally relates to a hybridoma cell line and the antibodies produced thereby, which are specific for said TAIC.

1.2 The documents mentioned in this communication are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc. In addition, the additional document D9: WO 00/42856 (by The Schepens Eye Research Institute) is cited, a copy of which is enclosed.

1.3 The present application satisfies the criterion set forth in Article 33(2) PCT because the subject matter of claim 1-26 is novel in view of the cited prior art.

2. Inventive step (Article 33(3) PCT)

The subject matter of claims 1-26 involves an inventive step. None of the cited prior art documents suggests the cultivation of blood-derived monocytes with both M-CSF andγ-IFN, in order to provide transplant acceptance inducing cells.

3. Industrial applicability (Article 33(4) PCT)

The subject matter of claims 1-26 is industrially applicable.

Amended Set of Claims

1. A process for the preparation of transplant acceptance inducing cells of monocytic origin characterised in that

   a) monocytes are isolated from blood;

   b) the monocytes are multiplied in a suitable culture medium which contains the cellular growth factor M-CSF;

   c) the monocytes are cultivated simultaneously with or following step b) in a culture medium containing γ-IFN; and

   d) the transplant acceptance inducing cells formed in step c) are obtained by separating the cells from the culture medium.

2. A process according to claim 1 characterised in that the monocytes are of human origin.

3. A process according to claims 1 or 2 characterised in that the monocytes are isolated form the blood in such a manner that next to the monocytes also lymphocytes are present in an amount of at least 10% by reference to the total cell number in the isolate.

4. A process according to claims 1 to 3, characterised in that the transplant acceptance inducing cells formed in step c) or obtained in step d) are selected by binding to the antibody produced by the hybridoma cell line DSM ACC2542.

5. A process according to claims 1 to 4, characterised in that among the transplant acceptance inducing cells formed in step c) or obtained in step d) of claim 1 or obtained in the selection step according to claim 4 those cells are selected which co-express the antigens CD3 and CD14 on their cell surface.

6. A process according to claims 1 to 5, characterised in that the M-CSF concentration in the culture medium is 1 to 20 µg/l.

7. A process according to claims 1 to 6, characterised in that, subsequent to step b) the monocytes are cultivated for 24 to 72 hours in a culture medium containing γ-IFN, the cultivation in the presence of γ-IFN beginning 3 to 6 days after the beginning of cultivation step b).

8. A process according to claim 7, characterised in that the γ-IFN concentration in the culture medium is 0.1 to 20 ng/ml.

9. A process according to claims 1 to 8 characterised in that the total cultivation period in steps b) and c) is 4 to 8 days.

10. A process according to claims 1 to 8 characterised in that subsequent to step d) of claim 1, or subsequent to the selection steps according to claims 4 and 5, the cells are suspended in a suitable cell culture medium or in a PBS or NaCl solution.

11. A process according to claims 1 to 10 characterised in that the cells are suspended in a freezing medium and are subsequently deep frozen.

12. A process according to claim 11 characterised in that the freezing medium comprises fetal calf serum (FCS) or human AB serum and DMSO.

13. Transplant acceptance inducing cells of monocytic origin obtainable by any of the processes according to claims 1 to 12 characterised in that they co-express the antigens CD3 and CD14 on their cell surface.

14. Transplant acceptance inducing cells according to claim 13 characterised in that they are of human origin.

15. Cell preparation containing the transplant acceptance inducing cells according to claims 13 or 14 in a suitable medium.

16. Pharmaceutical composition containing transplant inducing cells of monocytic origin characterised in that they co-express the antigens CD3 and CD14 on their cell surface.

17. Pharmaceutical composition containing the transplant acceptance inducing cells according to claims 13 or 14 or the cell preparation according to claim 15.

18. Use of the transplant acceptance inducing cells according to claims 13 or 14 or the cell preparation according to claim 15 for manufacturing a pharmaceutical composition for the suppression of transplant rejection reactions.

19. The use of transplant acceptance inducing cells according to claims 13 or 14 or the cell preparation of claim 15 for *in vitro* generating and/or propagating regulatory T-lymphocytes.

20. The use according to claim 19, wherein the regulatory T-lymphocytes co-express the antigens CD4 and CD25 on their cell surface.

21. A process for the generation and/or propagation of regulatory T-lymphocytes, characterised in that

a) transplant acceptance inducing cells according to claims 13 or 14 or a cell preparation according to claim 15 are co-cultivated with a T-lymphocyte preparation, and

b) the regulatory T-lymphocytes are optionally obtained from the culture medium.

22. A process according to claim 21, characterised in that the regulatory T-lymphocytes co-express the antigens CD4 and CD25 on their cell surface.

23. A process according to claims 21 or 22, characterised in the regulatory T-lymphocytes are obtained from the culture medium by FACS sorting.

24. Hybridoma cell line DSM ACC2542.

25. Antibodies produced by the hybridoma cell line DSM ACC2542.

26. The use of the antibody according to claim 21 for the detection and/or selection of transplant acceptance inducing cells.